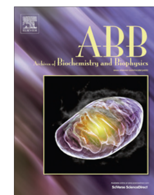
Archives of Biochemistry and Biophysics xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi



# Inactivation of human myeloperoxidase by hydrogen peroxide ☆

Martina Paumann-Page [a,b], Paul G. Furtmüller [b], Stefan Hofbauer [b], Louise N. Paton [a], Christian Obinger [b,*], Anthony J. Kettle [a]

[a] Centre for Free Radical Research, University of Otago Christchurch, Christchurch, New Zealand
[b] Department of Chemistry, Division of Biochemistry, BOKU – University of Natural Resources and Life Sciences, Muthgasse 18, Vienna, Austria

ARTICLE INFO

*Article history:*
Received 18 June 2013
and in revised form 2 September 2013
Available online xxxx

*Keywords:*
Myeloperoxidase
Neutrophils
Oxidative stress
Suicide inhibitor
Mechanism-based inhibition
Oxidative modification

ABSTRACT

Human myeloperoxidase (MPO) uses hydrogen peroxide generated by the oxidative burst of neutrophils to produce an array of antimicrobial oxidants. During this process MPO is irreversibly inactivated. This study focused on the unknown role of hydrogen peroxide in this process. When treated with low concentrations of $H_2O_2$ in the absence of reducing substrates, there was a rapid loss of up to 35% of its peroxidase activity. Inactivation is proposed to occur via oxidation reactions of Compound I with the prosthetic group or amino acid residues. At higher concentrations hydrogen peroxide acts as a suicide substrate with a rate constant of inactivation of $3.9 \times 10^{-3}\ s^{-1}$. Treatment of MPO with high $H_2O_2$ concentrations resulted in complete inactivation, Compound III formation, destruction of the heme groups, release of their iron, and detachment of the small polypeptide chain of MPO. Ten of the protein's methionine residues were oxidized and the thermal stability of the protein decreased. Inactivation by high concentrations of $H_2O_2$ is proposed to occur via the generation of reactive oxidants when $H_2O_2$ reacts with Compound III. These mechanisms of inactivation may occur inside neutrophil phagosomes when reducing substrates for MPO become limiting and could be exploited when designing pharmacological inhibitors.

© 2013 The Authors. Published by Elsevier Inc. All rights reserved.

## Introduction

Neutrophils are the predominant white blood cells in circulation. They are highly specialized for their primary function, the phagocytosis and destruction of invading pathogens by antimicrobial proteins and reactive oxidants [1]. When stimulated, neutrophils consume oxygen in a respiratory burst that produces superoxide and hydrogen peroxide [1]. Simultaneously, these white blood cells discharge the abundant heme enzyme myeloperoxidase (MPO)[1] that uses hydrogen peroxide to oxidize chloride, bromide and thiocyanate to the respective hypohalous acids and hypothiocyanite. These oxidants kill ingested bacteria but are also implicated in tissue damage associated with numerous inflammatory diseases [2]. It has been demonstrated that during phagocytosis the amount of extractable neutrophilic MPO decreases while a significant fraction of the soluble enzyme is inactivated [3,4]. The mechanism by which this occurs and its relevance to infection and inflammation have yet to be investigated in detail.

Enzyme inactivation is likely to involve reactions with either hydrogen peroxide or its products because they have the potential to oxidatively modify amino acids and the prosthetic heme group. It has been demonstrated with several heme peroxidases, including horseradish peroxidase [5,6], ascorbate peroxidase [7] and lactoperoxidase [8], that hydrogen peroxide alone can promote irreversible inactivation. There are also reports that MPO is inactivated by hydrogen peroxide in the absence of exogenous electron donors [9,10]. However, the mechanism of inactivation and the effect on its structural integrity have not been investigated.

In this study we aimed to show how hydrogen peroxide affects the activity of MPO and describe the mechanisms involved in enzyme inactivation. We demonstrate that MPO is highly sensitive to inactivation by hydrogen peroxide. Irreversible inactivation of MPO was accompanied by changes in its secondary and tertiary architecture including breakage of covalent heme linkages and disruption of its subunit structure.

☆ This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

* Corresponding author. Address: Department of Chemistry at BOKU – University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria. Fax: +43 1 47654 6059.
*E-mail address:* christian.obinger@boku.ac.at (C. Obinger).

[1] *Abbreviations used:* MPO, myeloperoxidase; Por, porphyrin; TMB, 3,3′,5,5′-tetramethylbenzidine; DTPA, diethylenetriaminepentaacetic acid; cetrimide, alkyltrimethylammonium bromide; FOX, ferrous oxidation in xylenol orange; PVDF, polyvinylidene difluoride; CID, collision induced dissociation; ECD, electronic circular dichroism; DSC, differential scanning calorimetry; GdnHCl, guanidinium hydrochloride.

0003-9861/$ - see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2013.09.004

## Materials and methods

### Reagents

Human MPO (lyophilized and highly purified, Reinheitszahl 0.84) was obtained from Planta Natural Products (http://www.planta.at) and the concentration was determined spectrophotometrically with a molar extinction coefficient of 91,000 $M^{-1}$ $cm^{-1}$ per heme [11]. Hydrogen peroxide (30% analytical grade) was purchased from Biolab (Aust) Ltd. and concentrations were determined spectrophotometrically using a molar extinction coefficient of 43.6 $M^{-1}$ $cm^{-1}$ at 240 nm [12]. TMB (3,3′,5,5′-tetramethylbenzidine), xylenol orange, D-sorbitol, diethylenetriaminepentaacetic acid (DTPA), alkyltrimethylammonium bromide (cetrimide), glucose, catalase from bovine liver, bovine superoxide dismutase, glucose oxidase from *Aspergillus niger*, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt hydrate (ferrozine) and L-ascorbate were purchased from Sigma. Dimethylformamide and ferrous ammonium sulfate were from J.T. Baker.

All spectrophotometric assays were performed on an Agilent 8453 diode array spectrophotometer. For enhanced chemiluminescence Amersham™ ECL Plus Western Blotting Detection System from GE Healthcare was used.

### Myeloperoxidase activity assays

The residual peroxidase activity of MPO was determined by measuring its ability to oxidize TMB. MPO (32 nM) in 50 mM sodium phosphate buffer, pH 7.4, and 100 M DTPA was incubated at various protein to hydrogen peroxide ratios and aliquots were taken over time to assess the decline in peroxidase activity. Superoxide dismutase was added to a separate number of experiments at a concentration of 20 g/mL to investigate the requirement for superoxide in enzyme inactivation caused by hydrogen peroxide. Residual peroxidase activity was measured by adding a 25 μL aliquot to 850 μL of 200 mM sodium acetate buffer, pH 5.4, containing 0.01% cetrimide, 100 μL of 20 mM TMB in DMF (made fresh each day and kept in the dark) and 25 μL of 8 mM hydrogen peroxide. Reactions were performed at 28 ± 0.5 °C and started by addition of the MPO aliquot. TMB oxidation was followed at 670 nm and initial rates were calculated over the first 60 s of the reaction.

The halogenation activity of MPO was determined under the same conditions as described above with the exception that 10 mM bromide was present in the TMB assay. Under these conditions, bromide was the preferred substrate for MPO and was converted to HOBr, which was responsible for 80% of the oxidation of TMB with the remainder due to direct oxidation by MPO (i.e., peroxidase activity).

### Determination and generation of hydrogen peroxide

Consumption of hydrogen peroxide by MPO was measured using ferrous iron-catalyzed oxidation of xylenol orange (FOX assay) [13]. The FOX reagent was composed of 1 mM ammonium ferrous sulfate, 400 μM xylenol orange and 400 mM D-sorbitol in 200 mM $H_2SO_4$. Each peroxide assay was performed by adding 70 μL of sample to 25 μL FOX reagent while vortexing. The solution was then incubated at room temperature for 45 min in the dark prior to reading the absorbance at 560 nm. The hydrogen peroxide concentration was calculated against a standard curve of the range of 0–5 nM hydrogen peroxide. Samples with concentrations higher than the standard curve range were diluted accordingly in 50 mM phosphate buffer, pH 7.4, before adding to the FOX reagent. This assay was also used to determine the rate at which glucose oxidase and glucose (1 mg/ml) produced hydrogen peroxide in 50 mM phosphate buffer, pH 7.4. The flux of hydrogen peroxide was linear over 60 min and increasing concentrations of glucose oxidase (0.1–2.5 μg/mL glucose oxidase) gave a linear increase in production of hydrogen peroxide (9.4 μM–285.5 μM per h).

### Spectral analysis of myeloperoxidase

Spectra of 1.5 μM MPO in 50 mM phosphate buffer, pH 7.4, containing 100 μM DTPA were recorded after reactions were started by adding hydrogen peroxide. To determine how peroxidase or halogenation substrates influenced the degradation of the heme groups of MPO, 200 μM ascorbate or 5 mM bromide plus, 5 mM methionine, respectively were also added as indicated below. The involvement of superoxide was checked by adding 20 μg/mL SOD to a separate set of reactions.

### SDS–PAGE analyses

To follow the impact of hydrogen peroxide on the structural integrity of myeloperoxidase, 1.5 μM MPO in 50 mM sodium phosphate buffer, pH 7.4, and 100 μM DTPA was incubated with 1.5 mM hydrogen peroxide. Aliquots were taken at 5, 10, 25, 40 and 60 min and residual hydrogen peroxide was removed by adding 20 μg/ml catalase. Samples of 15 μL were added to non-reducing and reducing sample loading buffers, respectively (final concentrations: 2% SDS, 10% glycerol, 125 mM Tris–HCl buffer, pH 6.5, and for reducing SDS–PAGE 1% β-mercaptoethanol). Samples were loaded without prior heating and resolved by 8–20% gradient SDS–polyacrylamide gel electrophoresis. Gels were stained with Coomassie Brilliant Blue R-250 for 60 min and subsequently destained. Gels were scanned using ChemiDoc® XRS (Bio-Rad).

Furthermore, 1.5 μM MPO in 50 mM sodium phosphate buffer, pH 7.4, and 100 μM DTPA was incubated with the following hydrogen peroxide: protein ratios: 1:1, 5:1, 10:1, 40:1, 100:1, 167:1, 500:1, 1000:1 and 2000:1 for 2 h at room temperature. Samples were resolved on SDS–PAGE, stained and analyzed as described above.

For detection of intact heme covalently linked to the protein, MPO was incubated with hydrogen peroxide and resolved by SDS–PAGE under the same conditions as described above and subsequently blotted onto a PVDF membrane (100 V, 60 min). Enhanced chemiluminescence (Amersham™ ECL Plus Western Blotting Detection System, GE Healthcare) was used to detect covalently bound and intact heme [14].

### Analysis of free iron

Release of free iron from the heme prosthetic group of MPO was measured colorimetrically using ferrozine following a published method but with slight modifications [15]. After buffer exchange with 50 mM sodium acetate buffer, pH 7.4, using a Micro Bio-Spin chromatography column (Bio-Rad) 1.5 μM MPO was treated with 1.5 mM hydrogen peroxide and incubated at room temperature for 2 h. The volume of 1 mL was reduced to dryness and the pellet resuspended in 30 μL water. Ascorbic acid (30 μL of 1.13 mM in 0.2 M HCl) was added and left for 5 min. The protein was then precipitated by adding 30 μL of 11.3% trichloroacetic acid and samples were kept on ice for 5 min followed by a short fast spin at 4 °C. Finally, 36 μL of 10% ammonium acetate was added to the supernatant followed by 9 μL of 6.1 mM ferrozine and the absorbance at 563 nm was measured after 5 min ($\varepsilon$ = 28,000 $M^{-1}$ $cm^{-1}$).

UNCORRECTED PROOF

Download English Version:

https://daneshyari.com/en/article/8290533

Download Persian Version:

https://daneshyari.com/article/8290533

Daneshyari.com